# SULPHURIC ACID AS A WEED SPRAY [1]

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## HISTORICAL REVIEW

### EARLY WORK

One of the most striking methods of weed control is the use of selective sprays which will kill the weeds without injury, or with but slight injury, to the growing crop. These sprays are used for killing annual weeds in grain crops. When this method was first accidentally discovered, in 1896, by the French grape grower, L. Bonnet, it aroused great interest throughout the agricultural world, with the result that in the following years more or less extensive experiments were carried out.

In the United States Bolley (*7*),[3] who was the earliest worker to try out weed sprays, and he obtained excellent results. He states: "Farmers have been backward in applying what the writer believes to be one of the most effective methods for aiding the cereal producer in eradicating weeds." So successful were Bolley's experiments that he wrote: "The gain to the country at large * * * will be much greater in monetary consideration than that which has been afforded by any other single piece of investigation applied to field work in agriculture." However, after his experience in 1900 Bolley (*6*) changed his opinion to some extent. That year was dry, and it was shown that "these weeds [mustards, etc.] do not always die down by the treatment so easily, indeed that in dry slow-growing periods spraying should not be attempted." Bolley used several sprays, including soldium chloride, iron sulphate, copper sulphate, corrosive sublimate, and sodium arsenite.

Several other workers, Pammel and King (*28*), Moore and Stone, (*23*), Stone, (*36*), and others obtained similar results. The success of the spraying was connected rather closely with prevailing conditions for growth and weather during and after the spray had been applied. Schultz (*34*), Bornemann (*8*), Wehsarg (*39*), and other German authors described extensive experiments with various chemical sprays in Germany, where the method was utilized more than in any other part of the world. Extensive experiments are also reported from England and Scotland, since workers were very eager to try the new method of weed eradication. Brenchley (*9*) summarizes several of these experiments.

Many countries now possess a rather voluminous literature dealing with weed sprays. Sulphate of iron was the chemical used chiefly

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[3] Reference is made by number (italic) to "Literature cited," p. 1089.

or exclusively. The results obtained are various and often contradictory. Under favorable conditions the results were excellent, but failures caused by rains or other complications are often reported.

## A NEW PERIOD

In the earlier experiments various chemicals, usually solutions of salts, were used as sprays. Acids do not seem to have been tried to any great extent, and when used the results seem to have been no more satisfactory than with other sprays. For instance, Woods (*41, p. 100–101*) in 1904, reports that a 20 per cent solution of iron sulphate reinforced with 5 per cent sulphuric acid failed to destroy *Raphanus raphanistrum*. Experience indicates that *R. raphanistrum* is very easily killed by either sulphuric acid or iron sulphate. It is possible that Woods was working with another similar plant, possibly *Brassica campestris*.

However, in 1911 Rabaté (*29*) reports some results from experiments on weed eradication in winter wheat, which mark the beginning of a new period. He used solutions of copper sulphate, iron sulphate, and sulphuric acid. He concludes that sulphuric acid in 6, 8, or 10 per cent solutions, the degree of strength depending upon local conditions, is the most satisfactory spray to use. The solution was applied at a rate of 1,000 liters per hectare (107 gallons per acre). It killed most annual weeds but did not retard the growth of the wheat, although the lower leaves were killed. The sulphuric acid solution also had a fertilizing effect on the soil. Later on Rabaté (*30, 31*), as well as other workers in France, reported similar results. Jaguenaud (*18*) found that sulphuric acid killed wild radish, wild mustard, crowfoot, vetches, and vetchling without injury to wheat. He used 7 liters of sulphuric acid (66° Baumé) to every hectoliter of water, which gave approximately a 10 per cent solution. Several papers deal with the eradication of weeds in flax (*17, 19, 32*). Morettini (*24*) in 1914–15 reports similar results in Italy. In sprayed fields he obtained an increase of 6 bushels of grain per acre.

In the Scandinavian countries, especially in Norway, the new method of weed eradication is now extensively used. Korsmo (*20, 21, 22*) started very comprehensive experiments on weed eradication in 1914. The chief spray he used was a diluted sulphuric acid. In spring-sown grain he found a strength of 3.5 to 4 per cent solution applied at a rate of 1,000 liters per hectare (107 gallons per acre), sufficient to kill all annual weeds to which the spray could adhere. As a result he obtained a very marked increase in yield. For instance, for the average of 211 experiments carried out from 1914 to 1922 in spring-sown grain crops, he obtained an increase of 490 kgm. of grain (25.3 per cent above unsprayed plots) per hectare. Calculated per acre the increase in yield of grain would be about 430 pounds.

Unfortunately, the results are published only in Norwegian, so that they are almost unknown outside of Scandinavia. In 1921 the writer (*1, 2, 4*) began experiments on weed eradication in Sweden under conditions similar to those of Korsmo, and has obtained very satisfactory results.

## WEEDS REPORTED KILLED BY SULPHURIC ACID

Table 1, listing weeds reported killed by sulphuric acid, is compield from the reports of several workers.

TABLE 1.—*Weeds reported killed by sulphuric acid solution of different concentrations*

| Name of weed | Investigator reporting | Percentage concentration of sulphuric acid solution used |
|---|---|---|
| *Adonis aestivalis* L. (summer adonis, or pheasant's-eye) | Morettini | 10.0 |
| *Anthemis arvensis* L. (corn camomile) | Korsmo | 3.5 to 4.0 |
| *Anthemis cotula* L. (May weed or dog fennel) | do.<br>Rabaté | 3.5 to 4.0<br>10.0 |
| *Brassica alba* (L.) Boiss. (white mustard) | Korsmo | 3.5 to 4.0 |
| *Brassica arvensis* (L.) Ktze. (wild mustard or charlock) | Korsmo and others | 3.5 to 10.0 |
| *Brassica campestris* L. (rutabaga) | Korsmo | 3.5 to 4.0 |
| *Capsella bursa-pastoris* Med. (shepherd's purse) | do.<br>Rabaté | 3.5 to 4.0<br>10.0 |
| *Centaurea cyanus* L. (bachelor's button) | Korsmo | 3.5 to 4.0 |
| *Chenopodium album* L. (pigweed, lamb's quarters) | Åslander | 3.5 t0 4.0 |
| *Chenopodium rubrum* L. (red goosefoot) | Korsmo | 3.5 to 4.0 |
| *Chenopodium polyspermum* L. (pigweed or lamb's quarters) | do | 3.5 to 4.0 |
| *Chrysanthemum segetum* L. (yellow oxeye) | do | 3.5 to 4.0 |
| *Cuscuta sp.* (dodder) | Rosa (*33*) | 4.0 to 5.0 |
| *Daucus carota* L. (wild carrot) | Morettini | 10.0 |
| *Delphinium consolida* L. (larkspur) | Korsmo | 3.5 to 4.0 |
| *Erophila verna* E. Mey | Rabaté | 10.0 |
| *Erysimum cheriranthoides* L. (wormseed mustard) | Korsmo | 3.5 to 4.0 |
| *Euphorbia peplus* L. (petty spurge) | do | 4.0 |
| *Fagopyrum tartaricus* Gaertn. (Tartarian buckwheat) | do | 3.5 to 4.0 |
| *Galeopsis tetrahit* L. (hemp nettle) | do | 3.5 to 4.0 |
| *Galeopsis speciosa* Mill | do | 3.5 to 4.0 |
| *Galium aparine* L. (cleavers) | do | 3.5 to 4.0 |
| *Lamium amplexicaule* L. (henbit) | do | 3.5 to 4.0 |
| *Lamium purpureum* L. (dead nettle) | do | 3.5 to 4.0 |
| *Lapsana communis* L. (nipplewort) | do | 3.5 to 4.0 |
| *Lathyrus spp.* (wild peas) | Jaguenaud | 10.0 |
| *Lepidium campestre* (L.) R. Br. (field peppergrass) | Korsmo | 3.5 to 4.0 |
| *Lepidium ruderale* L. (peppergrass) | do | 3.5 to 4.0 |
| *Matricaria discoidea* DC. (pineappleweed) | do | 3.5 to 4.0 |
| *Matricaria chamomilla* L. (wild chamomile) | Jaguenaud | 10.0 |
| *Papaver argemone* L | Korsmo | 3.5 to 4.0 |
| *Papaver dubium* L. (poppy) | do | 3.5 to 4.0 |
| *Papaver rhoeas* L. (field poppy) | do<br>Rabaté | 3.5 to 4.0<br>10.0 |
| *Polygonum aviculare* L. (knotweed, or knotgrass) | Korsmo | 3.5 to 4.0 |
| *Polygonum convolvulus L.* (black bindweed, or wild buckwheat) | do | 3.5 to 4.0 |
| *Polygonum lapathifolium* Ait. (smartweed) | do | 3.5 to 4.0 |
| *Pteris aquilina L.* (bracken, or brake fern) | Gordon (*14*) | 5.0 |
| *Ranunculus arvensis* L. (buttercup) | Rabaté<br>Morettini (*25*) | 10.0<br>10.0 |
| *Ranunculus ficaria L.* (lesser celandine) | Rabaté | 10.0 |
| *Ranunculus* spp. (buttercups) | Jaguenaud | 10.0 |
| *Raphanus raphanistrum* L. (wild radish) | Korsmo | 3.5 to 4.0 |
| *Senecio vulgaris* L. (groundsel) | do | 3.5 to 4.0 |
| *Sisymbrium officinale* (L.) Scop. (hedge mustard) | do | 3.5 to 4.0 |
| *Sisymbrium sophia* L. (flixweed) | do | 3.5 to 4.0 |
| *Solanum nigrum* L. (black nightshade) | do | 3.5 to 4.0 |
| *Sonchus oleraceus* L. (sow thistle) | do | 3.5 to 4.0 |
| *Specularia speculum* Alph. DC. (Venus's-looking-glass) | Morettini | 10.0 |
| *Spergula arvensis* L. (corn spurry) | Korsmo | 3.5 to 4.0 |
| *Stellaria media* (L.) Cyrill. (chickweed) | do | 3.5 to 4.0 |
| *Thlaspi arvense* L. (penny cress, French weed) | do | 3.5 to 4.0 |
| *Urtica urens* L. (nettle) | do | 3.5 to 4.0 |
| *Vicia* spp. (vetches) | Jaguenaud | 10.0 |
| *Viola tricolor* (hearts ease) | Korsmo | 3.5 to 4.0 |

Table 1 shows a wide range of concentration of sulphuric acid used. Korsmo never used more than a 4 per cent solution and obtained excellent results. In France and Italy a 10 per cent solution is commonly claimed to be necessary for the complete destruction of the weeds. This difference probably is due chiefly to the fact that Korsmo has been working in spring-sown crops, while the later data are obtained in fields of winter wheat sprayed in February or March. Rapidly growing plants in spring-sown crops are much more susceptible than winter annuals in wheat. This point will be fully discussed later. This apparent difference in the susceptibility of plants may also be due in part to the different types of spraying machines employed. Korsmo had a machine constructed especially for spraying sulphuric acid, which worked very satisfactorily. The possibility of killing a certain weed by a dilute sulphuric-acid solution depends on the amount of spray actually adhering to the plant, which in turn depends to a great extent upon the type of spraying machine used. The writer's experiments on weed eradication have confirmed the importance of a good spraying machine in obtaining good results. That the importance of the proper spraying machine has sometimes been overlooked is clear when a writer recommending a 10 per cent solution declares that the spray can be distributed by a watering can.

## WEEDS REPORTED BADLY INJURED BY SPRAY OF SULPHURIC ACID

Certain weeds are not killed by sulphuric-acid spray. Among these are many perennial weeds the leaves of which may be destroyed while the roots are unharmed, thus allowing new shoots to appear soon. Spraying of growing crops is not directed against perennial weeds but against annual, winter annual, and possible biennial weeds. Table 2, which lists some of the weeds reported badly injured by sulphuric-acid spray does not therefore, embrace all weeds known to be harmed. This does not mean that sprays, especially sulphuric-acid sprays, may not be of some importance in combating some perennial weeds. For instance, it is the writer's experience that spraying an oat field at the proper time will prevent shoots of *Cirsium arvense* (Canada thistle) from flowering. Dehn (*13*) states that the best method of eradicating weeds in lawns is to apply some drops of sulphuric acid on the crown of each plant.

TABLE 2.—*Weeds reported badly injured by spraying with sulphuric-acid solution*

| Name of weed | Investigator reporting | Percentage concentration of sulphuric acid solution used |
|---|---|---|
| *Adonis flammea* Jacq | Rabaté | 10 |
| *Agrostemma githago* L. (corn cockle) | do | 10 |
| *Centaurea cyanus* L. (bachelor's button) | do | 10 |
| *Lathyrus aphaca* L. (wild pea) | do | 10 |
| *Lathyrus hirsuta* L. (wild pea) | do | 10 |
| *Scandix pecten-veneris* L | do | 10 |
| *Vicis angustifolia* Reichard (vetch) | do | 10 |
| *Vicia cracca* L. (wild vetch) | do | 10 |

## WEEDS NOT INJURED BY SPRAYS OF SULPHURIC ACID

Several weeds are reported as not injured by sulphuric acid. A waxy surface and the concealing of the easily injured growing point protects the grasslike weeds as well as the grain plants. Dense hairs or glandular hairs protect some weeds against the sulphuric acid spray as well as against other sprays which act on the top of the plants. Several weeds have a smooth surface to which the spray can not adhere.

TABLE 3.—*Weeds reported not injured by spraying with sulphuric-acid solution*

| Name of weed | Investigator reporting | Percentage concentration of sulphuric-acid solution used |
|---|---|---|
| *Allium rotundum* L. (wild onion) | Morettini | 10.0 |
| *Allium viniale* L. (wild garlic) | do.<br>Voelcker (*37*) | 10.0 |
| *Alopecurus agrestis* L. (foxtail grass) | Rabaté | 10.0 |
| *Anchusa officinalis* L. (alkanet) | Korsmo | 3.5 to 4.0 |
| *Avena fatua* L. (wild oats) | Several workers | 3.5 to 10.0 |
| *Avena strigosa* Schreb. | Korsmo | 3.5 to 4.0 |
| *Bromus mollis* L. | do. | 3.5 to 4.0 |
| *Bromus secalinus* L. (chess or cheat) | do. | 3.5 to 4.0 |
| *Carduus crispus* L. | do. | 3.5 to 4.0 |
| *Cirsium lanceolatum* Scop. (bull thistle) | do. | 3.5 to 4.0 |
| *Erodium cicutarium* L'Her. (stork's-bill) | do. | 3.5 to 4.0 |
| *Euphorbia helioscopia* L. (wartweed) | do. | 3.5 to 4.0 |
| *Fumaria officinalis* L. (fumitory) | do. | 3.5 to 4.0 |
| *Lolium temulentum* L. (darnel) | do. | 3.5 to 4.0 |
| *Matricaria inodora* L. | do. | 3.5 to 4.0 |
| *Medicago spp.* | Morettini | 10.0 |
| *Muscari spp.* (grape hyacinth) | do. | 10.0 |
| *Ornithogalum spp.* | do. | 10.0 |
| *Senecio viscosus* L. | Korsmo | 3.5 to 4.0 |
| *Sonchus asper* L., Hill | do. | 3.5 to 4.0 |

Tables 1 to 3, inclusive, show that most weeds, not perennial, are killed when sprayed with sulphuric acid. Those unharmed are comparatively few and generally not so troublesome. These tables are not intended, however, to give the impression that sulphuric acid is the only spray which will kill these weeds. Under favorable conditions a solution of iron sulphate or copper sulphate may produce just as good results. Reports of experiments show, nevertheless, that the latter sprays often fail. Some of the reasons for this failure will be considered later in this paper.

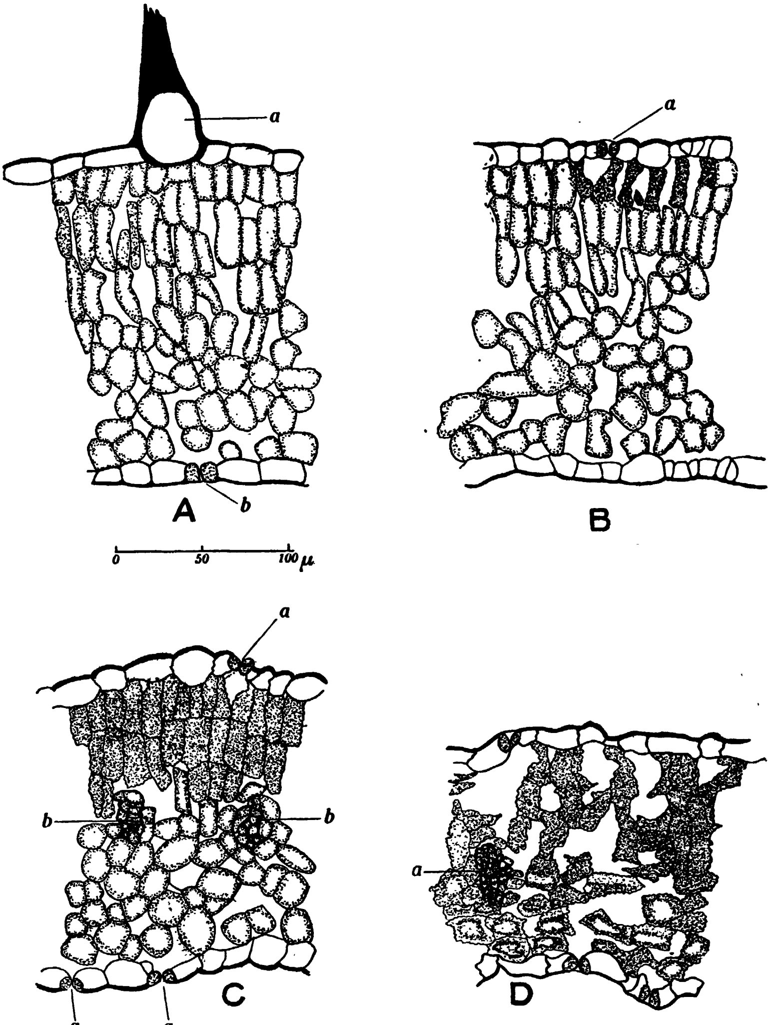
## PREVIOUS EXPERIMENTAL WORK

In hundreds of field trials it has been demonstrated that dilute sulphuric acid is an excellent spray. However, the action of the acid on plant tissues seems not to have been studied to any great extent. Recently the writer (*3*) has been able to show how sulphuric acts upon plants. Since a knowledge of this action is necessary for a further discussion, a brief summary is given here.

Several weeds and crop plants, grown in crocks in the greenhouse, as well as *Brassica arvensis* (mustard) growing in the field, were sprayed with diluted sulphuric acid solutions of various strengths. Because they were so hardy that they were not harmed by weak sprays the action of the sprays on mustard plants growing in the field was very easily studied. Cross sections of the sprayed and unsprayed leaves were made by the paraffin method. These sections were then examined microscopically to determine the action of the sulphuric acid upon the tissues of the leaves. The most striking action of the sulphuric-acid spray was as follows:

(1) After adhering to the surface of the plants it penetrates very rapidly and kills the protoplasm almost instantly. Leaves of mus-

tard have stomata on both sides (fig. 1, A). It was found that the acid at first penetrates through the stomata, destruction of the cells being first noticeable in the neighborhood of these openings; but it




[F]IG. 1.—The action of sulphuric acid on plant tissues: (3) A, section of leaf of *Brassica arvensis* plant grown in the field during the winter (in England): *a*, hair on upper epidermis; *b*, stoma. B, section of leaf of field-grown plant one hour after it was sprayed with a 3 per cent solution of sulphuric acid: *a*, stoma through which the acid has penetrated and killed some cells. C, leaf similar to B, but showing the effect of the acid three hours after spraying: *a*, stoma; *b*, vascular bundles, diagrammatic. The acid has killed the upper half of the leaf, but the amount of the spray applied was not sufficient to kill the whole leaf. D, section of leaf three hours after it was sprayed with a 5 per cent solution of sulphuric acid: *a*, vascular bundle, diagrammatic. The whole leaf has collapsed as the amount of spray exceeded the quantity which the leaf was able to withstand. Note that the epidermis is the least injured part of the leaf

also seems to penetrate through the epidermal cells, as destruction was soon noticed in cells between the stomata. Figures 1 to 4 show the gradual increase of destruction caused by the penetrating acid.

(2) The sulphuric acid spray decomposes the chlorophyll as it unites with the magnesium atom of the chlorophyll molecule. (Fig. 1, B.) In order to prove this action of the acid, a few drops of 1 per cent solution of sulphuric acid were added to an alcoholic extract of chlorophyll from nettle leaves. It was found that four drops of this acid to five c. c. of chlorophyll extract immediately changed the chlorophyll color from deep green to yellowish green. This change in color indicates that the chlorophyll was split into phaeophytin and magnesium sulphate. According to Willstätter (*40*) this reaction may be indicated by the formula:

$$C_{55}H_{72}O_5N_4Mg + H_2SO_4 = C_{55}H_{72}O_5N_4H_2 + MgSO_4, \text{ or}$$
$$\text{Chlorophyll} + \text{sulphuric acid} = \text{phaeophytin} + \text{magnesium sulphate}$$

Only a very small amount of highly diluted acid is necessary to bring about this reaction, since the amount of chlorophyll in leaves is less than 1 per cent of the dry weight.

(3) It breaks up the chloroplasts. In sections of unsprayed leaves and in cells not yet affected by the acid the chloroplasts were plainly visible. In cells penetrated by the acid the cell contents formed a deeply stained mass in which no chloroplasts could be seen. (Fig. 1, C.) Apparently the acid breaks up the structure of the plastids.

(4) The acid does not destroy the cell walls, at least not those of the epidermis. Sulphuric acid is generally known to be very corrosive. It would seem then that it would "burn" the cell wall as it does clothes and other objects. But Figure 1, A to D, inclusive, shows that the epidermal cells are the least injured parts of the sprayed leaves. This may be explained by the fact that sulphuric acid of low concentration does not dissolve cellulose, which is regarded as the principal constituent of the cell walls. (Fig. 1, D.) The cell walls of the spongy parencyhma of sprayed leaves of pot-grown plants sometimes seemed to be destroyed by the spray. These delicate cell walls are supposed to be built up mostly of pectin (or pectin in combination with calcium) compounds which are dissolved by weak solution of sulphuric acid. However, the destruction of these leaves after the spray was applied was so rapid and complete that the fate of the cell walls was difficult to determine.

Further, it was found that plants grown in the greenhouse were easily destroyed by a 2 per cent solution while plants in the field which had been growing during the winter (in England) required no less than a 5 per cent solution to kill them. The same quantity of spray was used in all cases. As the acid can not evaporate, the plants must be able to absorb a certain amount without being harmed. Death follows spraying only when the sprayed quantity exceeds this amount.

Analyses of the plants showed that the field-grown plants had a far greater amount of dry matter, especially of ash, than the greenhouse-grown plants. This suggested that up to a certain point some of the constituents of the ash were able to neutralize the acid.

It was further found that the leaves of the greenhouse-grown plants changed in anatomical structure with their height above the cotyledons. They became more compact up to the fifth or sixth leaf, the number developed during the time of the investigations.

In reports on spraying experiments it is frequently stated that weeds are most difficult to kill when sprayed in the late rosette stage. If the leaves become more compact in anatomical structure from the cotyledons upward it perhaps would be possible to explain their resistance by changes in anatomical structure accompanied by an increased amount of ash and dry matter. Further work is needed, however, to clear up this point.

Observations clearly indicate that grain crops are unharmed by a spray of sulphuric acid. Their resistance is due to a cutin layer which prevents the sprays from adhering to the plants. The concealed growing point is an additional protection, as several workers have pointed out. In field experiments it is always observed that the ends of the leaves of the grain plants are "burned," the day after spraying has been performed. The lower parts of the leaves are generally unharmed. The writer (*3*) has found this due to the fact that the cutin layer on the lower surface of the leaves is less protective than that on the upper surface. When the leaves of the grain plants have reached a certain size the ends turn over, so that the lower surface comes uppermost. Hence, this part of the leaf is hit by the spray and destroyed. The larger the grain plants are when sprayed the greater is the killed portion of the leaf. In windy weather the sprays will adhere more easily to the lower surface of the leaves and injure the crop.

In some crock cultures (*3*) a 10 per cent solution was sprayed on oats and barley without causing more harm than the weaker solution, as only a very small amount adhered to the plants. It was further found that the amount of spray per unit of area had some influence on the injury. When the commonly used quantities were sprayed only a few leaves were harmed; if larger amounts were used more leaves were injured. The smaller the grain plants and the more vertical their growth the less was the injury.

Peas (*Pisum sativum*), and red clover (*Trifolium pratense*), are found to be uninjured by sprays of sulphuric acid. The writer (*3*) has pointed out that red clover is protected against the sulphuric acid by dense hairs on the leaves. However, the cotyledons are unprotected and therefore are injured. Field trials have shown that a spray of sulphuric acid in a grain field, in which clover seed has been sown, does not harm the clover plants provided they have developed some true leaves. The leaves of peas are rather waxy, so that the spray does not adhere to them.

In some water cultures where seedlings of barley and beans were placed in a full nutrient solution, to which were added increasing amounts of sulphuric acid, the writer (*3*) found a concentration of the acid of 1:20,000 did not injure the plants. Weaker solutions seemed to stimulate the growth. (See fig. 2.)

## EXPERIMENTAL WORK

The experiments here reported were conducted to determine the influence of various environmental factors, and of the structure and composition of the plants, upon the effectiveness of sulphuric acid as compared with a solution of iron sulphate when used as weed sprays.

## MATERIALS AND METHODS OF PROCEDURE

Plants of field mustard, *Brassica arvensis* (L.) Ktze., and Cornellian oats, *Avena sativa* L., were grown in glazed crocks filled with soil. When the plants had grown to the desired size, they were sprayed

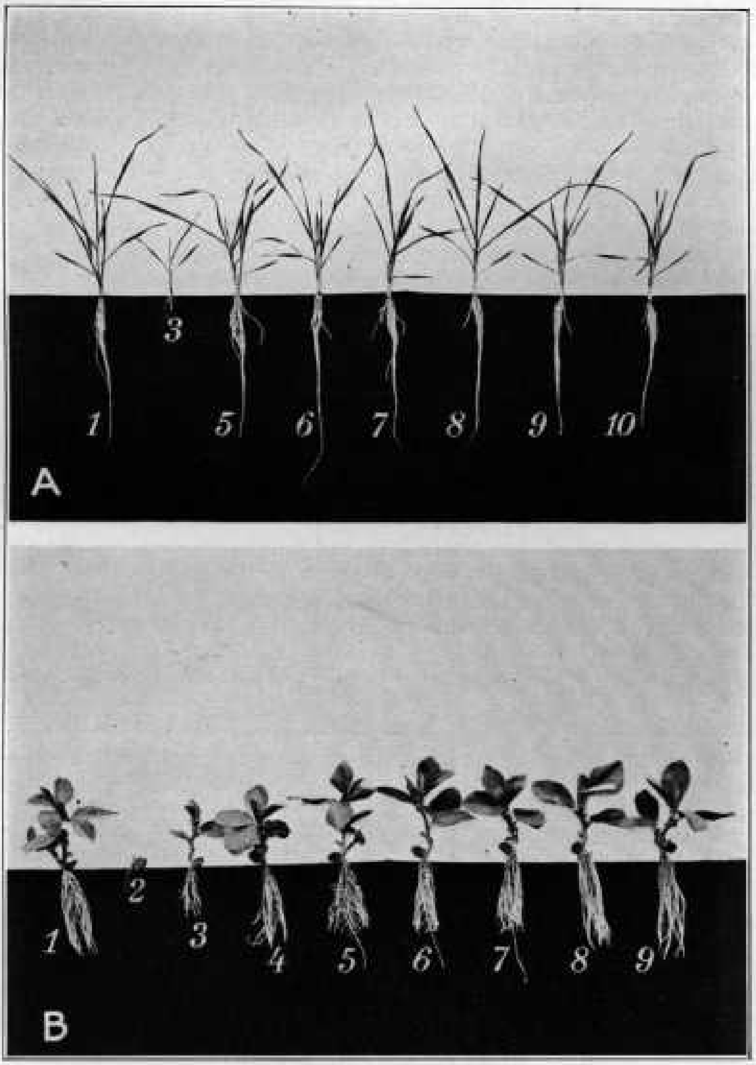



FIG. 2.—Effect of sulphuric acid on plant growth: A, Plants of barley grown in full nutrient solution to which were added increasing amounts of sulphuric acid; B, plants of beans (*Vicia faba*) grown in solutions similar to those in A

Amount of sulphuric acid in solution: 1, 0:1 (no acid); 2, 1:2,500; 3, 1:5,000; 4, 1:10,000; 5, 1:20,000; 6, 1:80,000; 7, 1:320,000; 8, 1:1,128,000; 9, 1:5,120,000; 10, 1:20,480,000

The concentration 1:20,000 had no injurious effect on the plants; weaker solutions seem to have stimulated the growth

with a solution of iron sulphate and dilute sulphuric acid and thereafter placed in glass chambers, where the atmosphere was kept at varying degrees of saturation. Observations were then made on the effect of the sprays on the plants. The leaves of mustard from some of the crocks were analyzed for dry matter and ash content. Samples of leaves were embedded in paraffin for study of structure.

In order to test the influence of the water content of the soil on the effect of the sprays, plants were grown under three conditions of soil moisture content. For this purpose glazed gallon crocks filled with sifted silt loam, into which had been mixed 10 per cent coarse sand, were used as culture vessels. The crocks held 4 kgm. of soil. The moisture content of the soil, at the time the crocks were filled, was found to be 8.3 per cent in the first experiment, and when the experiment was repeated 12.1 per cent.

In order to secure a uniform distribution of water throughout the soil, a special method was devised for watering the crocks (*5*). The water was applied through a small flower pot placed in the upper soil of the crock. From the flower pot the water was distributed laterally through four radiating "arms" of coarse sand which were inserted about 1 inch below the top of the soil. This method provided a very uniform distribution of soil moisture.

The seeds of mustard and oats were sown February 8, 1926. The plants in each crock were thinned to four oat plants and eight mustard plants. In the repeated experiment the plants were sown on March 22.

In order to obtain an even germination, all of the cultures were at first watered uniformly with a sprinkling can. This method was continued until the cotyledons were well developed. After this the cultures were divided into three lots and the water content was maintained at 15, 30, and 45 per cent of the water-holding capacity of the soil as determined by Hilgard's (*16*) method. Table 4 shows the moisture of the soil of the series in the two experiments.

TABLE 4.—*Moisture content maintained in soil in culture crocks containing mustard and oats*

| Test series and experiment Nos. | Average original moisture content of soil | Average weight of water-free soil | Average water-holding capacity of soil | Moisture content of soil maintained during experiments | Quantity of water to be added to soil of original weight of 4 kgm. | Weight of soil plus moisture per crock |
|---|---|---|---|---|---|---|
| | *Per cent* | *Kgm.* | *Per cent* | *Per cent* [a] | *Gm.* | *Kgm.* |
| Test series No. 1: | | | | | | |
| Experiment 1 | | | | 15 | 70 | 4.070 |
| Experiment 2 | | | | 15 | [b] −10 | 3.990 |
| Test series No. 2: | | | | | | |
| Experiment 1 | | | | 30 | 460 | 4.460 |
| Experiment 2 | | | | 30 | 460 | 4.460 |
| Test series No. 3: | | | | | | |
| Experiment 1 | 8.3 | 3.668 | 41.9 | 45 | 860 | 4.860 |
| Experiment 2 | 12.1 | 3.516 | 47.2 | 45 | 930 | 4.930 |

[a] Indicated as percentage of water-holding capacity.
[b] Crock allowed to evaporate 10 gms. of water in order to come down to 15 per cent moisture.

The water content of the crocks was maintained at a uniform stage by keeping the weight of the crocks constant. As long as the plants were small, so that the water content changed but slowly, the crocks were weighed every second or third day depending on the temperature; but as the plants grew larger it was necessary to water them every day, especially those in medium moist and wet soil.

The average quantities of water lost by the different cultures varied. In experiment No. 1 of series 1, 2, and 3, for the period February 22 to March 14, the losses were 370, 950, and 870 gm., respectively. In experiment No. 2 the temperature was higher, so that the loss of water was greater. During the period April 4 to 22 the loss for experiment No. 2 in series 1, 2, and 3, was 380, 1,260, and 1,170 gm., respectively. These losses represent evaporation from the surface of the soil as well as transpiration by the plants.

As the season gave rather insufficient sunlight for growing strong plants, four 60-watt electric lights were mounted over the cultures and turned on from sunset until midnight.

The temperature of the greenhouse was held at 10° C. during the night. During the daytime the temperature rose depending on the amount of sunlight. Ventilation often proved insufficient to keep the temperature below 16° to 18° C. As a result the plants grew very rapidly and became more succulent than if they had been grown in the open.

## SPRAYS USED AND THEIR APPLICATION

The effect of a spray depends to a certain degree on its concentration. If its effect is to be studied the most reliable data may be obtained by the use of a solution of low concentration as differences are most easily observed with such a solution. For this reason, and as the plants were rather succulent, sulphuric acid in concentrations of 1, 1.5, and 2 per cent by weight was used in the present study. The iron-sulphate solutions used in experiment No. 1 were always five times stronger than the sulphuric-acid solutions with which they were to be compared, namely 5, 7.5, and 10 per cent. The writer's experience in field trials indicates that a 20 per cent solution of iron sulphate is about as effective a weed spray as a 4 per cent solution of sulphuric acid. In some of the later applications in experiment No. 2 the concentration of the iron sulphate was increased to 15 per cent.

The plants were sprayed when they had developed four leaves which quite generally is regarded as the best time for this operation. For applying the sprays two types of atomizers were used. Since the sprayers were mounted on graduated cylinders it was easy to determine the amount of spray used. As nearly as possible 1 gm. per square decimeter was applied, equivalent to 1,000 liters per hectare or 107 gallons per acre, which is the amount generally applied under field conditions. The sprayings were performed during the forenoon when the temperature of the greenhouse was about 16° to 18° C. Each culture to be sprayed was divided by a screen of cardboard into two equal parts. One side was sprayed with sulphuric acid and the other with iron sulphate solution.

In order to test the possible influence of the humidity of the air on the effect of the sprays, the sprayed plants were exposed to three different conditions of humidity: (1) to a moist chamber of 90 to 100 per cent relative humidity; (2) to a dry chamber of about 30 per cent relative humidity; (3) to a greenhouse room in which the relative humidity was kept at about 60 per cent. The chambers used in these experiments were the same as those described by Muenscher (*26*). The temperature of the chambers was kept low by shading. The humidity of the greenhouse room was regulated by sprinkling with water and by ventilation.

## EFFECT OF SPRAYS ON MUSTARD PLANTS WHEN APPLIED UNDER VARIOUS CONDITIONS

From the experiments made in spraying plants with a solution of iron sulphate the following general deductions may be made:

(1) If the humidity of the atmosphere is low, so that evaporation is rapid, white crystals are soon formed on the surface of the leaves. The epidermis under the crystals may show a natural green color or it may be blackened, depending upon the hardiness of the leaves. The blackening of the leaves increases until they are completely black and dry. The petioles are generally not affected until the leaf is completely destroyed, as the spray seems not to adhere so readily to them. If the spray is strong enough, however, both petioles and stems will be killed.

(2) If the humidity of the air is high enough to prevent or greatly retard evaporation, crystals are not formed. The surface of the leaves becomes black under the drops of the spray, and the plants become flaccid. This continues until the leaves are dead.

Plants sprayed with sulphuric acid of sufficient strength react as follows:

(1) The plants soon become flaccid.

(2) At about the same time yellow spots appear under the drops of the spray or after the water of the spray has evaporated, so that the spray seems to have disappeared. These yellow spots soon turn brown and increase in size until the whole leaf is discolored. At the same time the leaf tissues begin to dry up. Generally the petioles and the stems of the plants are affected as soon as the leaves. The spray seems to adhere very easily to these parts of the plants. (See fig. 3.)

### Influence of Soil Moisture on the Effect of the Sprays

Soil moisture affected the development of the plants and thus indirectly the effect of the sprays. In the soil with the lowest water content, 15 per cent of water-holding capacity, the plants grew slowly. The color of the plants was a deeper green than those in the other series. The leaves were smaller and the internodes were shorter than on plants grown in soil with higher moisture content, and the plants appeared sturdier and were more hairy. In soil with medium water content 30 per cent of the water-holding capacity, the plants grew largest. The color was light green and the plants appeared rather succulent. Plants grown in wet soil, 45 per cent of water-holding capacity, appeared very similar to those grown in the medium moist series, except that they were somewhat smaller.

This difference in habitat had a marked influence on the effect of the sprays. However, the effect of a spray of iron sulphate was closely dependent upon the prevailing relative humidity of the air. It will be described therefore when that factor is considered. The action of sulphuric acid was dependent upon the strength of the solution. A 1 per cent solution failed to kill the plants in any of the series. The leaves became more or less scorched. Scattered over the surface were smaller or larger spots of dead tissues. A 1.5 per cent solution killed the plants of the medium moist and wet soil, while plants grown in the dry soil required a 2 per cent solution to kill them. The amount of spray was always 1 gm. per square decimeter.

### Influence of the Relative Humidity of the Air on the Effect of Sprays

As stated before, the plants were exposed to various conditions of atmospheric humidity after they had been sprayed. The influence of the humidity was rather marked.

#### INFLUENCE OF RELATIVE HUMIDITY ON THE EFFECT OF IRON SULPHATE SPRAY

In the moist chamber where no evaporation took place, the relative humidity of the air being maintained at or near 100 per cent, the

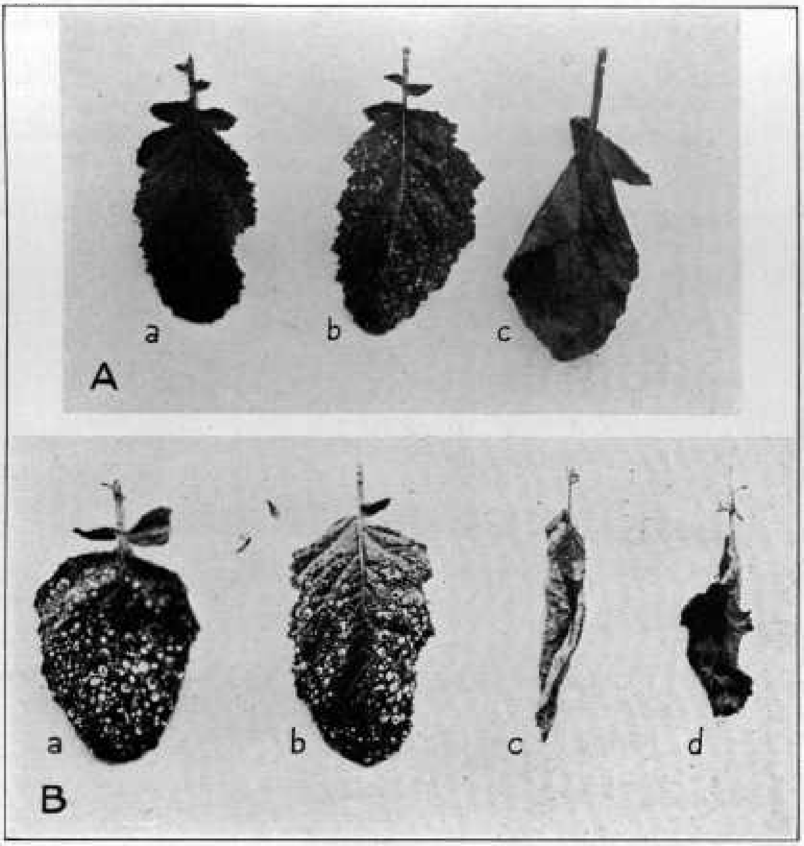



Fig. 3.—Effect of sprays on leaves of mustard: A, leaves one hour after spraying: *a*, unsprayed leaf; *b*, leaf sprayed with a 15 per cent solution of iron sulphate, on which crystals have formed, although the leaf is quite turgid; *c*, leaf sprayed with a 2 per cent solution of sulphuric acid, which was very flaccid and probably dead. B, leaves one day after spraying: *a* and *b*, leaves sprayed with iron sulphate, covered with crystals but still turgid; *c* and *d*, leaves sprayed with sulphuric acid which have dried and shriveled up

spray acted fairly rapidly. A blackening of the leaves under the drops of the spray was observed after four hours. The majority of the leaves were then more or less flaccid. After 24 hours the plants were completely destroyed. A 5 per cent solution proved strong enough to kill all mustard plants. (See fig. 4.)

With plants sprayed in the greenhouse, where the relative humidity was kept around 60 per cent, the effect of the spray was markedly different. As the spray evaporated, crystals appeared on the leaves.

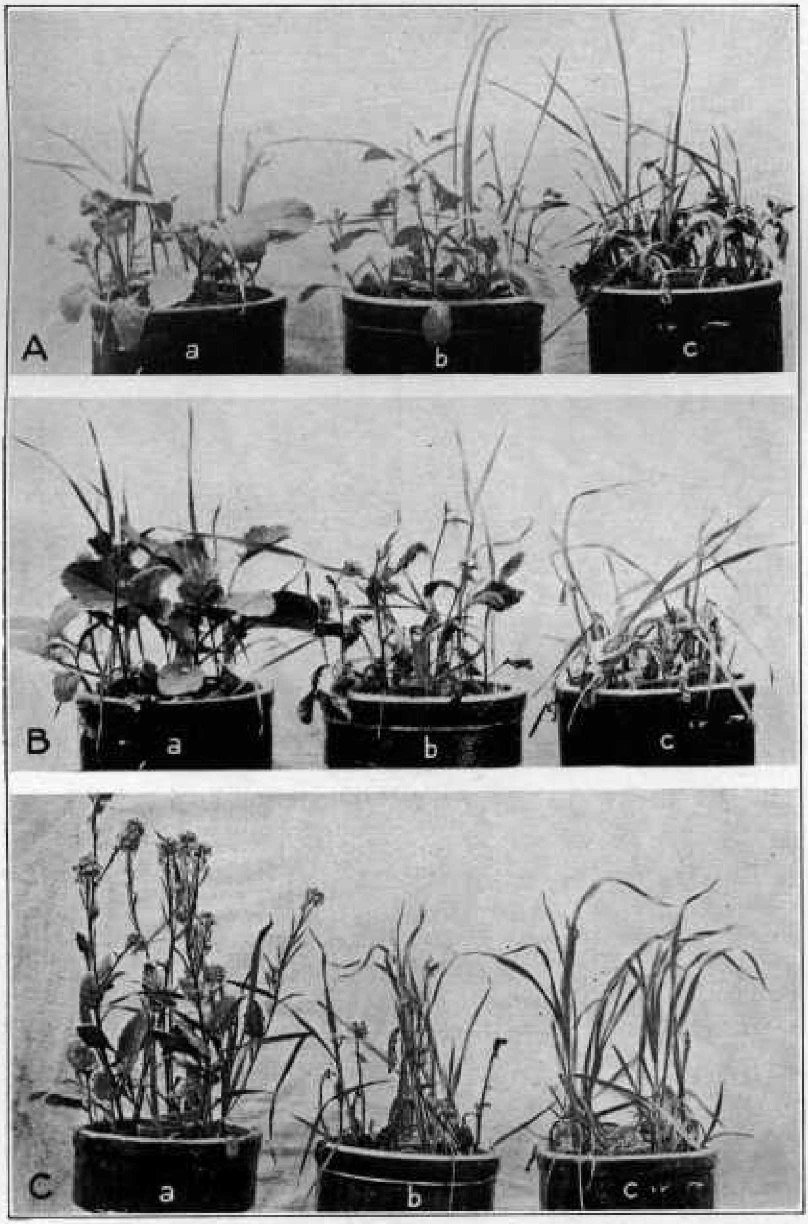



FIG. 4.—Influence of the relative humidity of the air on the effect of sprays. In each case (A, B, and C), *a* is the check crock containing plants which were unsprayed; *b* contains plants which were sprayed with a 15 per cent solution of iron sulphate; and *c* contains plants sprayed with a 2 per cent solution of sulphuric acid: A, appearance of plants one hour after spraying. Plants sprayed with sulphuric acid, crock *c*, are already dead, while the iron-sulphate spray has formed crystals on the leaves of the mustard plants without great damage to them. B, plants one day after spraying. Iron sulphate has affected the plants during the night when the relative humidity was high. C, plants one week after spraying. In the unsprayed pot, *a*, the mustard plants have completely outgrown the oat plants. The iron-sulphate spray has not been able to prevent some of the mustard plants from flowering, while the sulphuric-acid spray has absolutely killed the mustard without harming the oat plants, which are growing vigorously.

Plants grown in dry soil always showed perfectly unharmed leaves. The epidermis was green under the crystals and the leaves were turgid. Plants from medium moist soil appeared to be the most susceptible, as they frequently showed black spots under the crystals. However, the leaves rarely lost their turgor. After the crystals were formed, there seemed to be almost no increase in the injurious effect of the spray on the leaves. The crystals adhered rather loosely to the surface of the leaf. During the following night, when the relative humidity rose to 100 per cent, the action of the spray seemed to continue. By the next morning the effect was very marked, especially on plants grown in medium moist or wet soil. The surface of the leaves under the crystals was a deep black and the discoloration had reached the lower surface of the leaves. Where the crystals were close together, the whole leaf was black and dead. The plants of the dry soil showed greater resistance. Three to four days elapsed after spraying before they were completely destroyed.

In the dry chamber where the humidity was around 30 per cent, the effect of the spray was still less. Plants grown in dry soil were completely unharmed after 24 hours, in spite of numerous crystals covering the leaves. The plants of the moist and wet soil series were more susceptible to the spray and showed some slight injury.

### INFLUENCE OF RELATIVE HUMIDITY ON THE EFFECT OF THE SULPHURIC-ACID SPRAY

The action of sulphuric acid was very similar under all conditions of humidity under which it was applied. In the moist atmosphere the action was somewhat delayed, but after two hours exposure to a temperature of 20° C. the leaves were very flaccid and yellow spots appeared on 30 to 50 per cent of the leaf area. Petioles and stems were also affected similarly. On plants sprayed in the greenhouse the effect was visible much sooner, as the water from the dilute spray evaporated, so that the concentration of the acid increased. After a period of five to six hours the leaves were almost completely dried out. In the dry atmosphere the effect of the spray was visible sooner after its application. The leaves of the mustard plants were dead and dry after four hours.

## Influence of Temperature on the Effect of Sprays

In the second series of experiments an attempt was made to determine the influence of the temperature on the effect of the sprays. A 2 per cent solution of sulphuric acid and a 15 per cent solution of iron sulphate were used. As the experiments under higher temperature were carried out inside the greenhouse, and those under lower temperature were carried out in the open during cool days, the influence of the temperature was interfered with by the influence of the humidity of the air. However, under field conditions these factors are inseparable.

### INFLUENCE OF TEMPERATURE ON THE EFFECT OF IRON SULPHATE SPRAY

At an average temperature of 6° C. and a relative humidity of 78 per cent the action of a solution of iron sulphate had a greater effect than at 30° and a relative humidity of 52 per cent. At the lower temperature the spray did not evaporate in four hours, so that the crystals when formed had blackened the leaves. At the lower temperature the leaves frequently became flaccid and blackened, which rarely was the case at higher temperature, when the crystals were formed after 15 to 20 minutes.

### INFLUENCE OF TEMPERATURE ON THE EFFECT OF SULPHURIC-ACID SPRAY

The action of sulphuric acid was very markedly affected by the temperature. In medium moist soil, the action at the higher temperature, 30° C., was very rapid indeed. After 15 minutes the leaves began to wilt and small yellow spots appeared. In 30 minutes the petioles turned yellow and the stems began to bend over. After one hour the leaves were completely wilted and 50 per cent of the leaf area was yellow or brown. Four hours was time enough to dry the leaves. The plants in the dry soil were affected somewhat more slowly than those in the wet or medium moist soil, but after an hour the difference between the series was hardly detectable. At the lower temperature, 6° C., the action was much delayed. Wilting was not observed until two hours after spraying. Small yellow spots appeared at the same time. After five hours the effect was not quite so marked as after one hour at 30° C. After 24 hours the plants were dead but not dry.

## INFLUENCE OF RAIN ON THE EFFECT OF THE SPRAYS

Rain, falling after spraying is performed, may diminish or inhibit the effect of the operation, as the sprayed solutions will be washed off the plants. However, if the destruction of the plants by the sprays has proceeded beyond recovery when the rain occurs, the influence of the rain is negligible.

Experiments were conducted to test the time necessary for a spray to injure the plants beyond recovery. The plants were sprayed inside of the greenhouse and after a certain time, ranging from 30 minutes to 6 hours, sprinkled with a watering can. The temperature of the greenhouse was around 20° C. and the relative humidity around 55 per cent. It was found that plants sprayed with a 15 per cent solution of iron sulphate were almost unharmed when the spray was washed off three to six hours later. The leaves on plants grown in moist soil were blackened to some extent, but all plants recovered completely. Plants sprayed with a 2 per cent solution of sulphuric acid recovered when sprinkled 30 minutes after they had been sprayed, but if the sprinkling was done one hour after spraying the plants died. However, on the recovering plants, the top buds were destroyed, which means a great check to the plants.

### ADDITIONAL TEST OF THE EFFECT OF SOLUTIONS OF IRON SULPHATE AND SULPHURIC ACID ON PLANT TISSUES

In order to obtain additional evidence of the relative rapidity of the action of iron sulphate and sulphuric acid upon plant tissues the following experiments were performed with leaves of water weed, *Elodea canadensis* Rich. The easily observed streaming of the protoplasm in the leaf cells of *Elodea* gives a good indication of unharmed cells. Injury to the cells results in a cessation of the protoplasmic movement.

Leaves of the plant were mounted on cover glasses by using vaseline at one end. Hollow-ground slides such as are used in bacteriological work were used as containers for the solution to be tested, so that the leaf could be observed at any time during the experiment. The time of cessation of streaming was noted, after which the leaf was transferred to a solution of half the strength for half an hour and thereafter into water. After half an hour the leaves were reexamined and the effect noted. The following solutions were used: 10 and 20 per cent solutions of cane sugar, 5 and 10 per cent solutions of iron sulphate, and 0.5, 1, and 1.5 per cent solutions of sulphuric acid. The experiment was conducted at room temperature, about 20° C.

In a 10 per cent solution of cane sugar the streaming continued undisturbed for six hours. No plasmolysis was observed during this time. In a 20 per cent solution plasmolysis was induced and streaming ceased after about 20 minutes, probably owing to the increased viscosity of the cytoplasm. After the cells were transferred to a weaker solution of sugar and then to water they were deplasmolysed and some streaming was noted, indicating that the cells were unharmed. Cells were kept plasmolysed up to four hours with similar results. Plasmolysis did not harm the cells under these conditions.

In a 5 per cent solution of iron sulphate the cells did not become plasmolysed. The streaming began to decrease after about one hour but continued for about two hours. After the cells were placed in water they were tested in a 20 per cent solution of cane sugar. Some cells became plasmolysed after being in the solution of iron sulphate for three hours. The chloroplasts were still green but appeared massed together. A 10 per cent solution of iron sulphate caused plasmolysis after 10 to 15 minutes, but streaming continued up to two hours. The cells were not deplasmolysed when placed in water after they had remained in a 10 per cent solution of iron sulphate for three hours. The chloroplasts were of a natural color and size but clustered together in the plasmolysed cells.

Streaming was never observed when the cells were placed in a 1.5 per cent solution of sulphuric acid. The time necessary to place the slide under the microscope was apparently long enough to stop the movement. After a minute or more the chloroplasts became yellow. If the leaves were then placed in water and thereafter into a 20 per cent solution of cane sugar no plasmolysis was observed. In a 1 per cent solution of sulphuric acid the streaming was observed for 20 to 30 seconds. After two minutes the chloroplasts became yellow and the cells were dead. When the leaves were placed in a 0.5 per cent solution of sulphuric acid, streaming was observed for from two to four minutes. After five to seven minutes the chloroplasts were yellow and the cells showed no sign of plasmolysis when placed in a 20 per cent solution of cane sugar. In no case did a sulphuric-acid solution cause plasmolysis of the cells.

## ANALYSES OF PLANTS

In a previous study the writer (*3*) found a correlation between the chemical composition of plants and the strength of sulphuric acid necessary to kill the plants. It was also found that the farther away they were from the cotyledons the more compact was the structure of the leaves of pot-grown mustard plants. In order to determine whether the difference in the chemical composition of the later formed leaves was sufficient to explain the frequently reported hardiness of the plants in the late rosette stage, against sprays, leaves were analyzed for dry weight and ash content.

The mustard plants from five cultures of each series were harvested for analysis. As each culture had 8 plants, 40 plants of each series were analyzed. The green weight, dry weight, and total ash content were determined for the successive leaves of the plant beginning at the cotyledons. The results of these analyses are recorded in Table 5. This table shows that the plants in the dry soil had the largest amount of dry matter and ash, expressed in per cent of green weight.

TABLE 5.—*Analyses of Brassica arvensis plants in rosette stage, grown in greenhouse from February 8 to March 15, 1926*

[Figures represent total weight of 40 plants]

| Leaves analyzed | Green weight (grams) | Dry weight (grams) | Percentage dry weight | Ash (grams) | Ash in percentage of green weight | Ash in percentage of dry weight |
|---|---|---|---|---|---|---|
| Series 1 (plants grown in soil saturated to 15 per cent of water-holding capacity): | | | | | | |
| Cotyledons | 3. 75 | 0. 370 | 9. 87 | 0. 1155 | 3. 08 | 31. 21 |
| First leaf | 5. 5 | . 568 | 10. 32 | . 1575 | 2. 86 | 27. 72 |
| Second leaf | 8. 7 | 1. 122 | 12. 89 | . 246 | 2. 83 | 21. 92 |
| Third leaf | 6. 1 | . 994 | 16. 30 | . 166 | 2. 72 | 16. 70 |
| Series 2 (plants grown in soil saturated to 30 per cent of water-holding capacity): | | | | | | |
| Cotyledons | 8. 4 | . 534 | 6. 36 | . 185 | 2. 20 | 34. 64 |
| First leaf | 12. 9 | 1. 022 | 7. 92 | . 2655 | 2. 06 | 25. 98 |
| Second leaf | 23. 4 | 2. 017 | 8. 62 | . 475 | 2. 02 | 23. 55 |
| Third leaf | 22. 9 | 2. 238 | 9. 77 | . 4095 | 1. 78 | 18. 28 |
| Fourth leaf | 10. 0 | 1. 181 | 11. 81 | . 175 | 1. 75 | 14. 81 |
| Series 3 (plants grown in soil saturated to 45 per cent of water-holding capacity): | | | | | | |
| Cotyledons | 8. 9 | . 572 | 6. 62 | . 191 | 2. 14 | 33. 39 |
| First leaf | 11. 0 | . 956 | 8. 96 | . 241 | 2. 19 | 25. 31 |
| Second leaf | 17. 7 | 1. 615 | 9. 12 | . 3795 | 2. 14 | 23. 44 |
| Third leaf | 21. 8 | 2. 287 | 10. 48 | . 425 | 1. 95 | 18. 58 |
| Fourth leaf | 12. 0 | 1. 522 | 12. 68 | . 220 | 1. 83 | 14. 45 |

TABLE 6.—*Analyses of Brassica arvensis plants in rosette stage, grown in greenhouse from March 22 to April 22, 1926*

[Figures represent total weight of 40 plants]

| Leaves analyzed | Green weight (grams) | Dry weight (grams) | Per centage of dry weight | Ash (grams) | Ash in per centage of green weight | Ash in per centage of dry weight |
|---|---|---|---|---|---|---|
| Series 1 (plants grown in soil saturated to 15 per cent of water-holding capacity): | | | | | | |
| Cotyledons | 3. 5 | 0. 335 | 9. 57 | 0. 105 | 3. 00 | 31. 34 |
| First leaf | 4. 9 | . 537 | 10. 96 | . 128 | 2. 61 | 23. 83 |
| Second leaf | 7. 1 | . 852 | 12. 00 | . 172 | 2. 42 | 20. 18 |
| Third leaf | 6. 0 | . 806 | 13. 43 | . 144 | 2. 40 | 17. 87 |
| Fourth leaf | 2. 7 | . 382 | 14. 15 | . 059 | 2. 18 | 15. 45 |
| Series 2 (plants grown in soil saturated to 30 per cent of water-holding capacity): | | | | | | |
| Cotyledons | 9. 0 | . 536 | 5. 95 | . 172 | 1. 91 | 32. 08 |
| First leaf | 13. 8 | 1. 086 | 7. 87 | . 245 | 1. 78 | 22. 56 |
| Second leaf | 21. 9 | 1. 755 | 8. 01 | . 334 | 1. 52 | 19. 03 |
| Third leaf | 25. 1 | 2. 507 | 9. 98 | . 425 | 1. 69 | 19. 95 |
| Fourth leaf | 16. 4 | 1. 945 | 11. 86 | . 312 | 1. 90 | 16. 04 |
| Series 3 (plants grown in soil saturated to 45 per cent of water-holding capacity): | | | | | | |
| Cotyledons | 7. 8 | . 524 | 6. 72 | . 175 | 2. 24 | 33. 39 |
| First leaf | 12. 8 | 1. 080 | 8. 44 | . 247 | 1. 93 | 22. 83 |
| Second leaf | 19. 9 | 1. 780 | 8. 94 | . 396 | 1. 99 | 22. 25 |
| Third leaf | 20. 8 | 2. 252 | 10. 82 | . 391 | 1. 87 | 17. 37 |
| Fourth leaf | 13. 5 | 1. 630 | 12. 07 | . 241 | 1. 78 | 14. 78 |

TABLE 7.—*Amount of dry matter and ash per unit of leaf area of Brassica arvensis plants*

[Figures are based on 40 plants]

| Leaves analyzed | Area (sq. dcm.) | Dry matter (grams) | Ash (grams) | Quantity per sq. dcm. of leaf area of— Dry matter (grams) | Quantity per sq. dcm. of leaf area of— Ash (grams) |
|---|---|---|---|---|---|
| Series 1 (plants grown in soil saturated to 15 per cent of water-holding capacity): | | | | | |
| Cotyledons | 0.99 | 0.335 | 0.105 | 0.338 | 0.016 |
| First leaf | 1.42 | .537 | .128 | .378 | .090 |
| Second leaf | 3.16 | .852 | .172 | .269 | .054 |
| Third leaf | 3.16 | .806 | .144 | .255 | .045 |
| Fourth leaf | 1.10 | .382 | .059 | .347 | .054 |
| Total or average | 9.83 | 2.912 | .608 | .296 | .062 |
| Series 2 (plants grown in soil saturated to 30 per cent of water-holding capacity): | | | | | |
| Cotyledons | 1.98 | .536 | .172 | .271 | .087 |
| First leaf | 3.67 | 1.086 | .245 | .296 | .067 |
| Second leaf | 7.95 | 1.755 | .334 | .221 | .042 |
| Third leaf | 10.85 | 2.507 | .425 | .231 | .039 |
| Fourth leaf | 8.28 | 1.945 | .312 | .234 | .037 |
| Total or average | 32.73 | 7.829 | 1.488 | .239 | .045 |
| Series 3 (plants grown in soil saturated to 45 per cent of water-holding capacity): | | | | | |
| Cotyledons | 1.82 | .524 | .175 | .288 | .096 |
| First leaf | 4.00 | 1.080 | .247 | .270 | .061 |
| Second leaf | 6.70 | 1.780 | .396 | .265 | .059 |
| Third leaf | 7.96 | 2.252 | .391 | .283 | .049 |
| Fourth leaf | 6.52 | 1.630 | .241 | .250 | .037 |
| Total or average | 27.00 | 7.266 | 1.450 | .269 | .054 |

The plants in the medium moist soil showed the lowest content of dry matter and those in the wet soil an intermediate amount. However, the difference between plants of medium moist and wet soil was not very marked. Expressed in per centage of green weight, the dry matter of the leaves increased from the cotyledons upward while the ash content decreased.

Analyses of plants grown in the second experiment checked very closely with those of the first experiment. (See Table 6.)

The analyses of the plants in the first experiment gave results which show a marked difference in the chemical composition of the leaves, depending on their position above the cotyledons. But figures expressing percentages may give a false impression. If the action of sulphuric acid is diminished or inhibited by its absorption into the plant tissue (this action depends upon the amount of dry matter and ash of the leaves), it is obvious that it is the amount of dry matter and ash per unit of area which is important. The spray is always applied in a given quantity per unit area. Thus it was necessary to measure the leaf area in order to calculate the amount of dry matter and ash per unit of area. In the second experiment the leaf areas were determined by measuring tracings of the leaves with a planimeter. The results are recorded in Table 7.

Table 7 shows that there was a difference between the plants of the three series. Plants grown in the dry soil had the highest average amount of dry matter and ash per square decimeter of leaf area. Plants grown in medium moist soil had the lowest and those in wet soil had an intermediate amount of dry matter and ash. Thus far the figures agree with those expressing the amount in percentage of green weight. However, instead of increasing from the cotyledons upward, the dry matter per square decimeter actually decreases. The ash content also decreases, even to a more marked degree. The results of the analyses recorded in Table 7 seem to indicate that the resistance of the mustard plants, in late rosette stage, can not be due to the increased amount of dry matter and ash in the upper leaves of the plants.

## DISCUSSION

### THE EFFECT OF THE SPRAYS

The experiments show that there is a very marked difference in the action of the sprays used. The cells of a plant are able to endure a relatively high concentration of iron sulphate for a considerable time, while a very dilute solution of sulphuric acid kills the cells almost instantly. On the other hand, iron sulphate seems to be able to act for some distance. For instance, if plants are sprayed, the spray is seen to adhere to the leaves, but rarely to the petioles or the stems. Nevertheless, after some time, especially on plants placed in the moist chamber, it was found that both petioles and stems were black and killed. The salt seems to move through the tissues of the leaves. With sulphuric acid this was never observed, at least not with the quantities used as sprays, the acid acting solely at the place where it hits the leaf. On the other hand, it seems to adhere more readily to petioles and stems, as these were observed to be killed as rapidly as the leaves. It is shown, however, that sulphuric acid under certain circumstances may move through the leaves. If the tip of a leaf is placed in a solution of the acid, the whole plant will be killed. In the quantities applied as sprays the acid seems to be absorbed by the tissues without being transported to other parts of the plants.

The action of iron sulphate upon plant tissues is not clearly understood. Several theories have been advanced. Olive (*27*) believes that "death is due to osmotic properties rather than to absorption of the chemical into the leaves." The water, according to his theory, is drawn out of the leaves by the flakes of dried salt on the surface. He believes drying of the solution to be a necessary process. On the contrary, Schultz (*34*), citing some investigations by Stender (*35*), states that leaves are unharmed after being plasmolysed for several hours by various salt solutions, if the salt is then washed off. Earlier experiments by the writer (*3*) in which plant tissues were treated with strong solutions of sodium chloride, confirm these results. Schultz finds that iron sulphate has a certain harmful effect, but declares that he does not know how it acts.

The blackening of the leaves of sprayed plants is explained by Olive (*27*) as being due to the formation of sulphides in the leaf or "union of * * * sulphate with the living substance." Plants of the mustard family are characterized by the presence of mustard oils which contain a relatively high per cent of sulphur. This fact is probably the basis for Olive's interpretation of the action of iron sulphate. Schultz (*34*), however, denies that this reaction takes place. He believes that the blackening of the leaves is due to a reaction between iron and tannic acid of the tissues. Whatever the action may be, one thing is clear, and that is that the action is a slow one. Leaves of *Elodea* endured a 10 per cent solution for two hours.

The experiments show that a solution of iron sulphate may easily kill the tissues without causing plasmolysis. A 5 per cent solution completely destroyed the mustard plants in 24 hours in the moist chamber. But as such a solution did not cause plasmolysis in leaves of *Elodea*, even though most of the cells were killed within three hours, probably it acted in a similar way on leaves of mustard. In the moist chamber no evaporation took place, so that an increase in concentration was out of the question. The experiments thus support the view of Schultz that it is the chemical action of the spray that kills the plants.

The action of sulphuric acid is easier to interpret. It is the high hydrogen-ion concentration which is injurious. Brenner (*10, 11*) has studied extensively the effect of various acids on plant cells. He found them injurious in proportion to their acidity, i. e., in proportion to the actual hydrogen-ion concentration of the solution. He placed pieces of epidermis from several plants in acids of various strengths and tested their effect after a certain time. For instance, he found that a $\frac{M}{50}$ solution of sulphuric acid, equivalent roughly to a solution of 0.2 per cent killed cells of *Brassica oleracea* in two minutes. A $\frac{M}{1300}$ solution killed the cells in four hours. The hydrogen-ion concentration of this solution he gives as $1.4 \times 10^{-3}$, Expressed in a more common term of hydrogen-ion concentration, the acidity was approximately pH 2.85. Calculated in percentage this solution contained about 0.0075 per cent sulphuric acid. These figures, as well as the experiments with *Elodea* reported in this paper, show clearly that the protoplasm is very susceptible to an acid solution. However, it is not possible to destroy weed plants with a sulphuric-acid solution of 0.2 per cent strength, in spite of the fact that Brenner has shown that it will kill cells in two minutes. In these experiments a 2 per cent solution was necessary; in field trials in spring-sown grain crops a 3.5 to 4 per cent solution was required. Rabaté (*31*) found that a 10 per cent solution was just strong enough to kill weeds in winter wheat sprayed in early spring. This makes it clear that plants are able to absorb some acid and neutralize it, so that they will be killed only when the absorbing capacity has been exceeded. Only a small amount can adhere to the plants. The necessity of increased strength of spray when winter-grown weeds are to be eradicated indicates that the difference in resistance is to be looked for in the difference in anatomical structure of plants grown under various conditions. The illustrations in Figure 5 are cross sections of leaves of *Brassica arvensis* grown under various conditions. Plants grown under the most severe conditions have the most compact leaf structure. It is the cell walls which are affected. This makes it clear that the protecting capacity is situated in the cell walls, which seem to be able to absorb and neutralize at least some of the acid.

The great resistance of plants to sprays frequently observed in the late rosette stage, seems not, as far as these experiments show, to be due to the increased amount of dry matter and ash in the upper leaves, as earlier experiments suggested. There is actually a decrease in dry matter and ash content per unit of leaf area from the lower leaves upward. However, this increased resistance is reported, especially when sprays of salt solutions of various kinds have been used. Resistance to such sprays is a question to a large extent of the thickness and modification of the outer cell walls of the epidermis, which are greatly influenced by periods of drouth such as very commonly occur in spring and early summer. Still another explanation may be found. As long as the plants are small, the leaves are growing more or less horizontally. A spray at this time will adhere to the plants rather easily and in large amounts. When the plants grow larger and are in competition with grain plants the upper leaves grow more nearly vertically. A spray applied under these conditions adheres with more difficulty than on horizontal leaves and the amount of spray adhering per unit of leaf area will be less. The larger the leaves

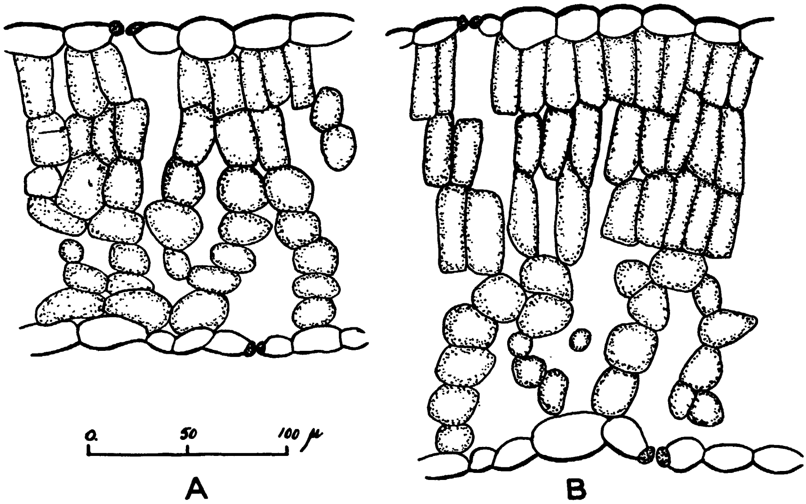



FIG. 5.—Cross section of leaves of *Brassica arvensis:* A, leaf of plant grown in medium moist soil, showing rather loose structure, weakly developed palisade tissue, and large air spaces; B, leaf of plant grown in dry soil, the structure of which is more compact, which accounts for the greater resistance of the plants to sprays

Both drawings were made with the aid of a camera lucida and are of the same magnification

of crowded plants, the more will these plants protect each other. Thus early spraying is always more destructive to the weeds.

## INFLUENCE OF WEATHER ON THE EFFECT OF SPRAYS

The experiments confirm the reports of field trials of weed eradication, namely, that rapidly growing plants are most susceptible to sprays. The most suitable weather conditions for spraying, according to general opinion, obtain on a dry, sunshiny, calm day. It is significant that Bolley (*6*) is the only one who recommends humid weather rather than dry weather for the application of weed sprays. Working in North Dakota he found the wettest and most rapidly growing condition to be the most satisfactory, and states that "it is useless to expect desirable results by spraying in droughty, windy weather." He used several salt solutions as sprays.

Experiments described in the present work fully confirm the statement of Bolley. The action of a solution of iron sulphate under dry conditions is very weak, especially on plants grown in dry soil. The salt crystallizes before the leaves are injured by the spray. In a moist atmosphere the spray acts rather efficiently. It is clear then that iron sulphate when sprayed under dry conditions begins to act when the relative humidity has increased to a certain point, which probably is near 100 per cent or at the dew point. For that reason good results may generally be expected in humid regions, and in dry regions only if the relative humidity happens to be high. With this point clear, it is of interest to examine the relative humidity in the agricultural regions of the United States during May and June, the time when weed sprays are usually applied. Day (*12*) in an extensive report on the relative humidity of the United States, shows very clearly that with the exception of the coastal regions, the relative humidity is very low during the spring and early summer.

Ward (*38*) in a paper discussing the relative humidity of the United States cites Hann (*15*), who states that the relative humidity of the New England States, one of the humid regions of the United States, is lower than it is in western Europe. He tries to explain this condition as an effect of prevailing winds. This low relative humidity explains why the use of iron sulphate and other salt sprays against weeds have given much better results in Europe than in the United States.

The action of sulphuric-acid spray upon plant tissues is favored by warm and dry weather. The warmer and dryer the weather the better are the results obtained.

The protoplasm is killed instantly when it comes in contact with sulphuric acid of the strengths used in sprays. The time elapsing from the moment the spray adheres to the plant until the tissues are killed depends upon the rate at which the acid penetrates the tissues. The rate of diffusion increases rather rapidly with rise in temperature. The fact that the rate of action of the spray increased about five times when the temperature was raised from 6° to 30° C. may be accounted for chiefly by the increased rate of diffusion. The action of the acid on the tissues is a chemical one. Rise in temperature increases the velocity of a chemical reaction still more than that of a physical one. Thus an increase in temperature accelerates the chemical action of the spray still more than its diffusion into the tissues. At the higher temperature employed in these experiments evaporation is very much higher than at the lower one. Evaporation of water from the spray increases the concentration of the sulphuric acid and thus increases its action. Another effect of the increased evaporation at the higher temperature is that the sprayed parts of the plant begin to dry up as soon as the protoplasm is killed, or as soon as the plant becomes flaccid. This drying rapidly increases the visible effect of the spray. Taking into consideration these effects of an increase in temperature on the action of the sulphuric-acid spray, its rapidly increasing effectiveness with rise in temperature is readily accounted for.

## SUMMARY

Plants of field mustard, *Brassica arvensis* (L.) Ktze, and Cornellian oats, *Avena sativa* L. were grown in pot cultures in the greenhouse.

The soil moisture of the cultures was kept at 15, 30, and 45 per cent of the moisture-holding capacity of the soil. A watering

system giving an even distribution of moisture in the soil was adopted. Mustard plants grown in the dry soil were found to have a much more compact anatomical structure than those grown in medium moist or wet soil.

The plants were sprayed with solutions of iron sulphate varying in strength from 5 to 15 per cent and with solutions of suphuric acid, varying in strength from 1 to 2 per cent. The sprayed amount was 1 gm. per square decimeter (1,000 liters per hectare, or 107 gallons per acre).

The sprayed plants were exposed to three conditions of atmospheric humidity, namely, about 30, 60, and 100 per cent of relative humidity. A solution of iron sulphate was found to be most destructive in an atmosphere containing about 100 per cent relative humidity. Under such conditions a 5 per cent solution completely killed the mustard plants in 24 hours. In dry air, with a relative humidity from 30 to 60 per cent, the solution of iron sulphate sprayed upon the plants evaporated rapidly and salt crystals were formed on the surface of the leaves without injury to the plants. When the relative humidity was allowed to increase to about 100 per cent, the plants were soon killed. This action was easily followed on plants grown in dry soil, while plants grown in moist soil were sometimes injured before the crystals were formed. Solutions up to 15 per cent strength were used without any different effect. As the relative humidity in the United States generally is very low during May and June a spray of iron sulphate will have but a slight effect on hardy plants.

When a solution of sulphuric acid was sprayed on the plants, the mustard plants were killed under all conditions of humidity, but best results were obtained in dry air. Plants grown in moist soil were killed off by a 1.5 per cent solution, while plants grown in dry soil required a 2 per cent solution to destroy them completely. This results indicates that the latter plants were able to absorb some acid without being injured permanently. As a spray of sulphuric acid gives the best results in dry air it is a spray to be recommended for dry regions.

Temperature had a marked influence upon the effect of the sprays. At 30° C. a 2 per cent solution of sulphuric acid killed the plants in one hour, while at 6° the same effect was obtained only after five hours. A 15 per cent solution of iron sulphate was more effective when sprayed at a lower temperature, as the evaporation of the water and crystallization of the sulphate was very slow giving the solution a longer time to act. In no case did a solution of iron sulphate kill the plants in less than 24 hours.

Artificial rain, produced by sprinkling, applied to the plants one hour after they had been sprayed with a 2 per cent solution of sulphuric acid failed to decrease the effect of the spray. Plants grown in moist soil sprayed with a 15 per cent solution of iron sulphate were but slightly harmed when "rain" was applied six hours after spraying. Plants grown in dry soil were unharmed.

In an additional test of the relative rapidity with which solutions of iron sulphate and sulphuric acid act upon living plant cells it was found that protoplasmic streaming in the leaves of *Elodea canadensis* Rich. continued for two hours in a 10 per cent solution of iron sulphate but ceased in 30 seconds in a 1 per cent solution of sulphuric acid. The cells were killed after having been kept in the iron sulphate solution for three hours or in the sulphuric acid for two minutes.

Analyses of plants indicate that the great resistance to sprays exhibited by plants in the late rosette stage, can not be explained by the increased amount of ash and dry matter in the upper leaves. Other explanations are suggested.

The oat plants were not harmed by the sprays.

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